

The Role of Adjuvant Immunotherapy in Renal Cell Carcinoma

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After nephrectomy for renal cell carcinoma (RCC), a significant number of patients develop recurrent disease. In order to improve the prognosis of these patients, the role of adjuvant immunotherapy should be clarified; the appropriate selection of patients is especially crucial. For this purpose, the search for prognostic factors is important to identify at-risk patients. Known factors such as stage, grade, and microvascular invasion can be used for appropriate selection. Other molecular markers, such as cadherin-6 and G250 antigen, may become important. So far, adjuvant immunotherapy in RCC has not shown improved survival data, but the results may be hampered by inadequate recruitment and follow-up. Adequate selection of patients and the search for less toxic and more effective immunotherapy approaches are of importance. Therefore, the use of monoclonal antibody G250 or dendritic cell vaccinations, alone or together with cytokines, may be advantageous and is currently used. Today, adjuvant protocols are open for recruitment of patients to elucidate the important question as to whether this approach should be implemented in the treatment of RCC. In this article, an update is given in the field of adjuvant immunotherapy in patients with RCC.

Introduction

Renal cell carcinoma (RCC) is the most common malignant disease of the adult kidney and accounts for 80% to 90% of all primary kidney neoplasms [1]. It is diagnosed in approximately 27,000 patients per year in the United States, and in 1997 approximately 11,300 Americans died of RCC [2]. From 1935 to 1989, the incidence of RCC has steadily increased in both females (from 0.7 to 4.2 per 100,000) and males (from 1.6 to 9.6 per 100,000) [3]. Overall, RCC is the tenth most common cancer and accounts for 3% of all adult malignancies [4]. The frequency of diagnosis increases above the age of 40 years, with a median age of 65 years. The disease is most prevalent in individuals of Scandinavian and

North American origin, and it is least prevalent in individuals of African and Asian origin [4]. Despite the increase in incidence, the mortality rate has decreased, indicating either that treatment has become more effective or that the disease is diagnosed in an earlier, better treatable stage [4]. Approximately 30% of all patients have metastatic disease at the time of diagnosis [5], and as many as 40% treated for their primary tumor will ultimately relapse with metastatic disease [6].

Because of the tendency of earlier diagnosis of patients with RCC and the high incidence of recurrent disease after local therapy, the ratio of adjuvant immunotherapy becomes a focus. The concept of adjuvant therapy is well known, and its relevance is relatively well established in such tumors as breast and colon cancer. Prerequisites for a successful adjuvant approach are twofold. First, there must be general acceptance of prognostic factors predicting the course of the disease, i.e., the risk of development of local recurrence and/or metastatic disease and death due to malignancy. These prognostic factors should preferably be easily detectable, reproducible, significant, and independent. Second, the availability of an active regimen is an important issue. These two prerequisites are discussed below.

Prognostic Factors

In general, the prognosis of patients with RCC with distant metastases is very poor. Patients with metastases experience an average survival of approximately 4 months, and only 10% of these patients survive longer than 1 year [7]. Patients who develop metastases following radical tumor nephrectomy (approximately 40%) have only a slightly more favorable prognosis, with a median survival time of approximately 11 months [7].

Lymph node involvement

The presence of nodal involvement is generally considered a poor prognostic sign in predicting the course of disease [8,9]. In a recent publication, it was shown that the probability of relapse was significantly higher in the node-positive category than in other patient categories [10]. In the node-positive group, 80% of patients relapsed within 30 months. Patients with node-negative disease had a much better prognosis, with only 40% relapsing at 3 years [11]. The reported incidence of nodal involvement in clinically nonmetastatic RCC shows wide variation and

depends greatly on the extension of the lymph node dissection [9].

In a study performed within the European Organization for Research and Treatment of Cancer Genito-urinary Tract Cancer Cooperative Group (EORTC GU), the number of node-positive patients who could be entered in an adjuvant study was very low and made continuation of such a study impossible. The fact that lymphadenectomy has no proven value in a randomized trial and the low incidence of node-positive disease in recent studies make it difficult to make lymphadenectomy mandatory as a staging procedure in the frame of an adjuvant study [12].

Stage, grade, and performance status

Additional prognostic factors are required to define a study group with a relatively high risk of relapse. Factors derived from primary tumor tissue are most attractive in view of the accessibility of this information. Anatomical extent is relevant, as is reflected in the fact that stage-related survival is independent of the staging system used, be it Robson or tumor, node, metastasis (TNM). A good example of further individualization of the treatment modalities with the use of prognostic factors is the recently proposed University of California Los Angeles Integrated Staging System (UISS) [13•]. The UISS includes the TNM staging system, the Fuhrman pathologic grading system and the Eastern Cooperative Oncology Group (ECOG) performance status. To validate this system, we evaluated our RCC database. The medical records of 207 patients with RCC (median age 60 years, median follow-up 30 months) seen at our kidney cancer clinic were evaluated. The patients were divided according to the proposed UISS staging system, and, subsequently, the 2- and 5-year survival of the different groups was estimated using the Kaplan-Meier method. Our patient characteristics are consistent with the UISS in terms of age, male-female ratio, and distribution of pathologic grading. These data confirm that, by using the UISS integrated staging system, five subgroups with different survival curves can be identified. These subgroups can be used for the inclusion in different protocols, including adjuvant treatments.

Microvascular invasion

The significance of microvascular invasion was studied in 217 patients who underwent a partial or radical nephrectomy [14••]. Fifty percent of patients with microvascular invasion without capsular invasion showed progression. Sixteen percent of patients without these features showed progression. Independent of capsular invasion, these figures were 61% and 12.4%.

The authors conclude that, in patients who underwent radical nephrectomy for clinically nonmetastatic RCC with microvascular invasion but without lymph node involvement or macroscopic vascular invasion, the chance of disease progression is estimated at 45% within 1 year. Microvascular invasion is the single most relevant prognosticator after presumed curative radical nephrectomy for RCC. Partly

based on these data, the EORTC GU group has started a randomized adjuvant study, which is discussed later.

Tumor markers

In order to select appropriate patients for new treatment options, it is of the utmost importance to use adequate tumor markers. Tumor markers may indicate the necessity for adjuvant therapy, but may also avoid these treatments and their associated toxicity. Markers that indicate the metastatic potential of RCC may be especially important to identifying at-risk patients. Two promising new markers are adhesion molecules and the G250 antigen.

Adhesion molecules

For RCC, the so-called cell adhesion molecule markers have been investigated. Cancer metastasis is a complex, multistage process. Decreased intercellular adhesion enables detachment of tumor cells and can play a role in the early steps of the metastatic process. Although cell adhesion can be mediated through at least four families of adhesion molecules (integrin, immunoglobulin, selectin, and cadherin), E-cadherin, a Ca^{++} -dependent epithelial cadherin, is considered to be a critical molecule for epithelial integrity [15]. Most RCCs, however, do not express E-cadherin because the renal proximal tubular epithelium from which RCCs originate does not express E-cadherin. In contrast, recent studies showed that in normal kidney tubular epithelium, both N-cadherin and cadherin-6 are expressed [16,17]. Thus, cadherin expression in RCC is more complex than in other carcinomas of the genitourinary tract.

Cadherin function is modulated through cytoplasmic proteins termed "catenins." Immunohistochemical staining revealed that catenins were expressed in all the segments of the nephron, including the proximal tubules. The catenin family seems to be less diverse than the cadherin family. Therefore, it was reasoned that there might be a correlation between the aggressiveness of RCC and a decreased expression of α -catenin, which is a member of the group of catenins that link cadherin to the cytoskeleton. Immunohistochemical staining of RCC using antibodies against E-cadherin and α -catenin have revealed that the ratios of abnormal staining were 77% and 37%, respectively. The prognostic value of E-cadherin is controversial. A significant correlation, however, between survival and decreased expression of α -catenin was observed. Whether α -catenin immunohistochemistry provides additional independent prognostic information remains to be established.

The G250 antigen

This antibody raised against human RCC has been shown to react with a large number of RCCs. Recently, G250 antigen was isolated and found to be homologous to the MN/CA9 gene originally identified in HeLa cells [18]. This protein is one of the best markers for clear cell RCC: all clear cell RCCs express this protein, whereas no expression

can be detected in a normal kidney and in most other normal tissue. Antibody studies have indicated that this molecule might also serve as a therapeutic target. By FISH analysis it was determined that the *G250/MN/CA9* gene is located on chromosome 9p12-13. To determine whether *G250* antigen (*MN/CA IX/G250*) could be a potential therapeutic target and a tumor marker, a total of 147 patients with RCC were investigated immunohistochemically as well as by reverse transcriptase polymerase chain reaction (RT-PCR) analysis. In addition, total RNAs extracted from patients' peripheral blood samples were analyzed for *MN/CA9/G250* mRNA signals. Immunohistochemistry demonstrated strong expression in 128 of 147 patients (87.1%), in contrast to the lack of expression observed in normal tissues. RT-PCR analyses of frozen specimens resulted in the clear detection of *MN/CA9/G250* mRNA signals in 137 of 147 patients (93.2%), and, despite subtle differences, the results were almost identical to those for immunohistochemistry. Although high-grade and -stage tumors exhibited significantly lower expression than low-grade and -stage tumors, a large proportion of tumors expressed *MN/G250* protein as well as mRNA. RT-PCR analysis of patients' blood samples revealed the presence of circulating monoclonal antibody *G250* (mAb *G250*)-expressing cells. These findings suggest that mAb *G250* may be a potential therapeutic target as well as diagnostic marker for RCCs. Tumors of low clinical stage showed a striking increase in *MN/CA9* expression, and high *MN/CA9* expression was associated with a good patient outcome. The results suggest that *MN/CA9* expression is a potential diagnostic biomarker of RCC, especially the clear cell type, and can be targeted using biomolecular techniques. These data were also confirmed by Murakami *et al.* [19].

Treatment Options for Adjuvant Immunotherapy in Renal Cell Carcinoma

For kidney-confined RCC, resection of the tumor, either by radical tumor nephrectomy or by nephron-sparing surgery (partial nephrectomy), remains the only curative option. As described above, however, a large number of patients present with metastatic disease at the time of diagnosis or develop metastases after surgery. The approach to disseminated RCC has changed significantly during the past decade and is still evolving. A review of 3502 patients with metastatic RCC treated with one of 72 chemotherapeutic agents revealed a cumulative objective response rate of only 6% [20]. Combination therapy with two or more agents does not significantly change response rates or survival [21]. Due to the low response rates, chemotherapy is now considered to be of limited value for the treatment of RCC. During the past decade, a number of therapeutic strategies have been developed to improve survival rate with manageable toxicity [22*]. These strategies include the use of biologic response modifiers (BRMs), such as interleukin-2 (IL-2), interferon- α (IFN- α), and adoptive

immunotherapy. Alternative approaches (eg monoclonal antibodies, gene therapy, or tumor vaccines) are still under investigation. The majority of studies investigating BRMs focused on the efficacy of IL-2 or IFN- α as single agents or in combination. The objective response rates in most of these trials were approximately 20% [22*]. Despite the initial promising results of cellular immunotherapy, such as lymphokine-activated killer cells or tumor infiltrating lymphocytes (TIL), this treatment modality showed disappointing response rates in phase III trials and is not considered effective for general treatment. More recently, several studies investigating the efficacy of BRMs together with other substances have reported substantially higher response rates. The initial experience with the combination of IL-2, IFN- α , and 5-fluorouracil (5-FU) was very promising, with response rates as high as 47%, but subsequent trials have failed to confirm such high response rates [23,24]. In a phase II trial with IFN- α combined with *cis*-retinoic acid, an objective antitumor response was observed in 30% of patients [25]. Again, a more recent study showed a substantially lower response rate of 17% for the combination of IL-2, IFN- α , and *cis*-retinoic acid [26]. Thus, although some new approaches to treat disseminated RCC look promising, larger phase III trials are required to establish the efficacy of these therapeutic regimens.

Adjuvant Protocols

In Italy, Pizzocaro *et al.* [27**] coordinated a multicenter randomized controlled trial to compare adjuvant recombinant IFN- α 2b with observation after radical nephrectomy for patients with stages II and III RCC. The first aim of this study was to evaluate whether, in an intention-to-treat context, adjuvant IFN could improve 5-year overall and event-free survival in patients with Robson stages II and III RCC after radical nephrectomy. The second aim was to investigate the role of prognostic factors in trying to identify possible subgroups of patients with different therapeutic responses in terms of event-free survival. This multicenter study included 1914 patients who underwent a radical nephrectomy and another 264 patients with Robson stages II and III. The two treatment arms were well matched for patient and tumor features. Treatment with IFN consisted of 6 million IU administered intramuscularly 3 times per week for 6 months starting within 1 month after surgery. It is important to know that 28% of the adjuvantly treated patients underwent a dose reduction because of toxicity. After a median follow-up of over 5 years, 89 patients relapsed, mainly in distant sites. Relapse occurred in 38 of the 124 control patients and in 51 of the 123 treated patients. Note that only four of the 38 relapsed control patients received IFN as secondary therapy. Eighty patients died: 39 in the control group and 41 in the IFN group. The overall survival probability at 5 years from surgery was, as estimated by the Kaplan-Meier method, 0.665 for the control group and 0.660 for the treated group. The

overall results of this study showed no advantage of adjuvant IFN therapy over observation in terms of overall and event-free survival.

Two more studies have been performed and concluded. The Delta-P Study Group performed a study of adjuvant therapy of RCC with IFN- α 2a and found no difference between observation and adjuvant treatment with IFN in terms of either time to treatment failure or survival [10]. ECOG performed a large randomized study on the same categories of patients, resecting high-risk RCC using lymphoblastoid IFN, and had similar results [28].

Most of these studies lack adequate recruitment and follow-up. This may be one of the most significant drawbacks of adjuvant protocols in RCC. Although there is a clear rationale for adjuvant immunotherapy, interest is lacking. In animal models, immunotherapeutic approaches were shown to be more effective in microscopic than in macroscopic established disease [29]. In patients with metastatic RCC, the highest response rates to cytokines are generally obtained in a situation with low tumor burden and no metastases to the CNS or bone [30,31].

This is why two studies addressing the role of adjuvant therapy have recently been initiated. The EORTC 30955 is studying adjuvant IL-2, IFN- α , and 5-FU for patients with high risk of relapse after surgical treatment for RCC. This randomized phase III study is a collaborative effort with the Medical Research Council from Great Britain and addresses the value of the triple combination therapy in patients without macroscopic tumors after radical surgery, emphasizing progression-free survival and overall survival. Moreover, the quality of life in the treatment and control groups is addressed.

The Working Party Immunotherapy Group is an American multicenter collaborative group that initiated a randomized phase III study addressing the role of high-dose IL-2 in the adjuvant therapy of high-risk RCC patients after nephrectomy.

Toward a More Specific and Less Toxic Immunotherapy Approach

In an effort to find a more effective and less toxic treatment for metastatic RCC, numerous combination regimens containing lower doses of IL-2 have been attempted. However, the combination of low dose IL-2 with other treatment modalities, such as INF- α [32], 5-FU [33,34], and TNF [35], have not been more efficacious than single-agent low-dose IL-2. Collectively, these results indicate that the IL-2-based treatment regimens that are currently available are limited by toxicity profiles or lack of tumor response. For the adjuvant immunotherapy setting, the search for less toxic strategies is crucial. Two promising strategies within this regard are described below.

Monoclonal antibodies

The concept of selective tumor targeting with antibodies is based on the avid interaction between the antibody and an

antigen, which is expressed on malignant cells but not on normal tissues. The term "tumor-specific antigen" should preferably be avoided since truly tumor-specific antigens have not been identified. Thus, a tumor-associated antigen can be defined as an antigen that is predominantly expressed by malignant cells of a certain tumor type. The most significant impulse in tumor targeting with antibodies came from Köhler and Milstein, who introduced the hybridoma technology [36], allowing the production of large quantities of identical, monoclonal antibodies with uniform immunoreactive behavior. In RCC, the cG250 is a IgG1- κ light-chain chimeric mAb that binds to a cell surface antigen found on 95% of clear cell renal cancer. A multicenter phase II study was performed to evaluate the safety and efficacy of repeated doses of mAb G250. For this purpose, 36 patients with metastatic RCC were included. All patients were nephrectomized for the primary tumor; target lesions were mostly lung lesions and lymph nodes. A weekly dose of 50 mg of mAb G250 was given by intravenous (IV) infusion for 12 weeks. Patients with stable disease or tumor response after 12 weeks of treatment could receive additional treatment for 8 more weeks. None of the 36 enrolled patients had any G250 grade III or IV toxicity. Only three patients had grade II toxicity, possibly related to the study medication (one had gastritis and two had vomiting). An enzyme-linked immunosorbent assay gave no evidence of human antichimeric antibodies. Nine patients presented with stable disease and were eligible for extension treatment. After the end of the study, in the follow-up period, one patient demonstrated a complete regression in week 38 (regression started in week 20) and another patient with stable disease showed a significant reduction of the overall tumor load in week 44, with gradual remission first observed in week 16. Both patients had multiple pulmonary target lesions. Five additional patients with progressive disease at study entry were stable for more than 6 months after the start of treatment; none of them received additional tumor therapy in the meantime. In conclusion, the weekly schedule of IV mAb G250 in patients with RCC was safe, very well tolerated, and nonimmunogenic in a 12-week treatment regimen. mAb G250 showed antitumor activity. This makes the approach ideal for adjuvant immunotherapy in patients at risk for the development of metastases. Such a trial is currently in preparation.

Dendritic cell vaccinations

Conventional therapy for metastatic cancer is limited by its lack of specificity, its associated toxicity, and its failure to cure most patients. New approaches to treatment are needed. The premise behind our work is that professional antigen-presenting cells, or dendritic cells (DCs), can be isolated from patients, armed with tumor antigens, and used to induce a specific antitumor response. DCs are bone marrow-derived leukocytes that express high levels of major histocompatibility complex (MHC) molecules; adhesion molecules; and other important costimulatory molecules such as B7-1 and B7-2, that are essential for the

process of antigen presentation [37,38]. DCs are the primary cells responsible for stimulating immune responses *in situ* [39,40], including antitumor immunity. Huang *et al.* [41] demonstrated that even immunogenic tumors, such as those modified to express B7-1, fail to stimulate the immune system, unless MHC-compatible antigen-presenting cells are available to process and present their antigens. By comparison, antigen-pulsed DCs are capable of stimulating a response simply by injecting them into naïve mice [42,43]. This effect is very potent and requires very few DCs, only 1 to 2×10^5 cells per mouse. Mayordomo *et al.* [44] recently reported that murine DCs pulsed with tumor peptides, and injected into recipient mice, induce antitumor immunity and complete recovery from pre-existing lethal tumor challenge. The clinical utility of DCs for treating human malignancies is just beginning to be explored. As mentioned, four patients with advanced B-cell lymphoma were recently treated with DCs that had been pulsed with their own idiotype protein [45]. This produced a complete remission in one patient, complete tumor regression in another, and measurable tumor reduction in a third. All of these results suggest that DCs have a unique and potent capacity to stimulate immune responses, including antitumor immunity.

It is so far unknown if transfecting DCs with a single antigen gives a better immune response than the transfection of multiple antigens. By using multiple antigens for transfection, one bypasses the fact that the great majority of tumor antigens are either unknown or indeterminate with regard to their immunogenic, cytotoxic T-lymphocyte epitopes. Multiple antigens can be obtained by oncolysis from tumor cell lines, and have been shown effective in DCs [46]. The advantage of a strategy with a monoantigen is the fact that there is no need for tumor tissue; it is purified, concentrated, and minimizes autoimmunity. In our institution, a phase I study was performed using tumor lysate-loaded DCs as vaccinations in metastatic patients after tumor nephrectomy. Although the feasibility of such a nontoxic approach was proven, no immunologic or clinical response was proven. In order to use more defined tumor antigens, a clinical study using G250 peptide-loaded DCs has been initiated and is currently ongoing at our institution.

Conclusions

The concept of adjuvant treatment in patients with RCC is still investigational. The rationale exists from animal studies and from the general concept of immunotherapy approaches. So far, the randomized studies performed have not shown a benefit of adjuvant immunotherapy over conventional treatments. The studies were hampered by inadequate recruitment and lack of adequate follow-up. Ideally, the design of adjuvant immunotherapy in RCC should be based on defining at-risk patients after local resection of renal tumors and acceptable toxicity profiles.

Prognostic factor research is crucial for this purpose. Known factors, such as stage, grade, and microvascular invasions, should be used for appropriate selection. Other molecular markers, such as cadherin-6 and G250 antigen, might become important in this setting. After adequate selection of patients, the search for less toxic and more effective immunotherapy approaches is next in importance. For this purpose, the use of mAb or DC vaccinations, alone or in combination with cytokines, may be advantageous and are currently ongoing.

In order to answer the important question of the role of adjuvant therapy in patients with RCC, adequate study design and recruitment are both necessary and demand large multicenter studies with adequate follow-up. It remains questionable if we will have this answer in the near future.

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